Development of a Poly(lactic acid) Poly(ethylene glycol) Nanoparticle for Delivery of Vitamin D_3 for Severe Asthmatics

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Introduction: Approximately 25% of all asthmatics are considered "severe" asthmatics, in that they do not respond to the standard treatment with albuterol (National Institute of Health). Compelling data suggest that 1,25 dihydroxyVitamin D_3 (Vit D) differentially modulates airway inflammation from that of GCs and may provide therapeutic advantages in the treatment of severe asthma [1-3].This project aims to synthesize nanoparticles containing a combination of 1,25 dihydroxyVitamin D_3 (Vit D) and albuterol. The Vit D is proposed as the novel anti-inflammatory drug, while the albuterol in this case will serve as the targeting moiety for the inflamed cells. In cases were the patient is still sensitive to albuterol, the two drugs are proposed to work syngerstically. The nanoparticle design is necessary to combine these two for controlled targeted local delivery. The backbone of the proposed micelle-like nanoparticle was made from a diblock copolymer of poly-lactic acid (PLA) and poly-ethylene glycol (PEG). PLA-PEG will self-assemble into micelle like structures that can be hardened into nanoparticles via solvent evaporation. The Vit D can easily be incorporated into the solid PLA core since it is hydrophobic, while the albuterol is chemically attached to the PEG end of the PLA-PEG copolymer. This allows it to be a readily available ligand for cell targeting, which is essential to keeping the nanoparticles in the lungs, therefore decreasing unwanted systemic side effects from either Vit D or albuterol.

Methods: Synthesis of the copolymer involved the ring opening polymerization of D,L-lactide to form PLA with PEG750 used as the hydrophilic end. The polymerization was catalyzed by tin(II) oxide in the presence of toluene. The block copolymer was then purified in selected solvents which were evaporated off and then analyzed with H^1NMR. The average molecular weight was determined through gel permeation chromatography (GPC). In order for attachment of Albuterol, a modified PEG polymer was used where an amine was added to one end group. The attachment of albuterol is completed through an alkylation reaction using $[Cp^*IrCl_2]_2$ as a catalyst. This reaction targets the 1^oalcohol of Albuterol and thus does not affect the drug's reactive sites. Cytotoxicity tests were conducted on unloaded PLA-PEG samples by inoculating human airway smooth muscle (HASM) cells with a small amount of the polymer and live/dead cells were counted after 2 days incubation. In order to demonstrate the vit D releases from the nanoparticles cells were treated with nanoparticle alone (- control), nanoparticle + Vit D and soluble Vit D (+ control). Nanoparticle was further divided into just the PLA-PEG backbone (nano) and PLA-PEG-Albuterol (nano-alb). ELISA's were performed to determine the transcription of 25-Hydroxyvitamin D 24-hydroxylase (CYP24A1), a gene commonly upregulated after Vit D stimulation.

Results: The synthesis of PLA-PEG copolymer was determined to be successful by H^1NMR. Scanning electron microscope images of the formed micelles conformed nanoparticle formation and showed an approximate diameter of 250nm. Particle sizing was further verified by DLS which showed average effective diameter of 280nm. When nanoparticles were loaded with Vitamin D, effective diameters increased to 400nm. Addition of surfactant was shown to stabilize nanoparticle size during formation. Cytotoxicity test showed a cell viability of 89% (+/-11%) of HASM cells after an inoculation of unload PLA-PEG and an incubation time of two days. Nanoparticles loaded with vitamin D were incubated with HASM cells and results of treatment with vehicle or either PLA-PEG or PLA-PEG-Alb with no Vit D had little effect on CYP24A1 transcription (Figure 1, - dosage of Vit D). However, both soluble Vit D and Vit D loaded nanoparticle (with or without albuterol) showed induction of CYP24A1 (Figure 1, 0.1 and 1 uM Vitamin D dosages) suggesting that both Vit D and the nanoparticle, but not PLA-PEG, activate the vitamin D receptor.

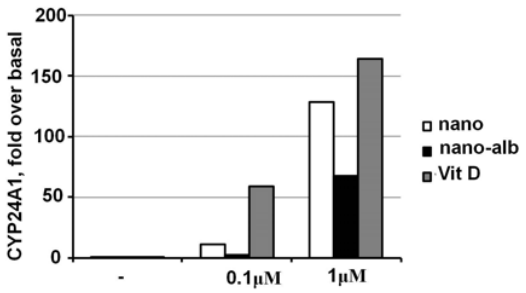

Figure 1. CYP24A1 induction by nanoparticle (either PLA_PEG(Nano) or nano+albuterol) and Vit D alone (+ control) at varying Vit D concentrations.

Conclusions: The PLA-PEG block copolymer was successfully synthesized and purified for HASM cell application, as verified by H^1NMR and the Vi-Cell analyzer. The molecular weight of the polymer averaged 6oo g/mol. SEM and DLS verified formation of the micelle-like nanoparticles consistent spherical shape and size. Additionally, HASM cells treated with the nanoparticle demonstrated transcription of CYP24A1 similarly to Vit D. Since it has been proven that synthesis of PLA-PEG nanoparticle is achievable and cell viability is good, optimizations of the copolymer as a delivery agent for Vitamin D in HASM warrants further studies.

References: 1.Damera, G., et al., *Vitamin D attenuates growth factor-induced human airway smooth muscle cell proliferation.* American Journal of Respiratory and Critical Care Medicine, 2009. **179**: p. A5606. 2.Damera, G., et al., *Vitamin D inhibits human airway smooth muscle proliferation through growth factor-induced phosphorylation of retinoblastoma protein and checkpoint kinase-1.* FASEB Journal, 2008. **submitted**.3.Litonjua, A.A. and S.T. Weiss, *Is vitamin D deficiency to blame for the asthma epidemic?* Journal of Allergy and Clinical Immunology, 2007. **120**(5): p. 1031-1035.